

What Is Claimed Is:

Suh  
E1

1. ~~A method for in vitro regeneration of poinsettia~~  
plants comprising the steps of:

- (a) incubating poinsettia plant tissue explants capable of producing reddish epidermal callus on callus induction medium;
- (b) subculturing reddish epidermal callus to embryo induction medium comprising casein hydrolysate to form embryogenic callus;
- (c) culturing said embryogenic callus on developmental medium;
- (d) culturing said embryogenic callus on maturation medium; and
- ~~(e) recovering poinsettia plants from said embryos.~~

2. The method of claim 1, wherein said callus induction medium comprises about 0.5 - 0.8 mg/liter 1-naphthalene acetic acid, about 0.2 - 0.4 mg/liter 6-benzylaminopurine and about 1 gm/liter casein hydrosylate.

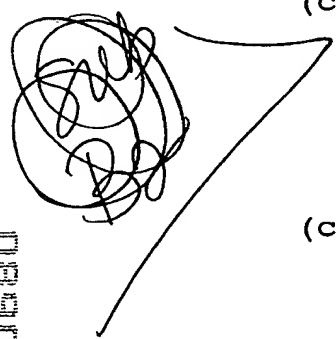
3. The method of claim 1, wherein said embryo induction medium comprises about 0.5-0.8 mg/liter 1-naphthalene acetic acid, about 0.2 - 0.4 mg/liter 6-benzylaminopurine 400 to 1700 mb/liter  $\text{NH}_4\text{NO}_3$ , 1900 to 3500 mb/liter  $\text{KNO}_3$  and about 1 gm/liter casein hydrosylate.

4. The method of claim 1, wherein said developmental medium comprises about 0.05 mg/liter 6-benzylaminopurine, and about 10 gm/liter mannitol.

5. The method of claim 1, wherein said maturation medium comprises about 5-20  $\mu\text{M}$  abscisic acid, about 30-100 gm/liter sucrose, about 1 gm/liter casein hydrosylate, and about 10 gm/liter mannitol.

26 FEB 20 11 46 AM '83

Sub  
22



467E20"46E0680

6. ~~A method for producing transgenic poinsettia plants, comprising the steps of:~~

- (a) incubating poinsettia plant tissue explants capable of producing reddish epidermal callus on callus induction medium;
- (b) culturing reddish epidermal callus on embryo induction medium comprising casein hydrolysate to form embryogenic callus;
- (c) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
- (c') introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene,
- (d) culturing said transformed embryogenic callus on selection medium;
- (e) culturing said transformed embryogenic callus containing embryos on developmental medium;
- (f) culturing said transgenic embryos on maturation medium; and
- (g) recovering transgenic plants from said ~~transgenic embryos.~~

7. The method of claim 6, wherein said callus induction medium comprises about 0.5 - 0.8 mg/liter 1-naphthalene acetic acid, about 0.2 - 0.4 mg/liter 6-benzylaminopurine and about 1 gm/liter casein hydrosylate.

8. The method of claim 6, wherein said embryo induction medium comprises about 0.5 - 0.8 mg/liter 1-naphthalene acetic acid, about 0.2 - 0.4 mg/liter 6-

benzylaminopurine and about 1 gm/liter casein hydrosylate.

9. The method of claim 6, wherein said developmental medium comprises about 0.05 mg/liter 6-benzylaminopurine, and about 10 gm/liter mannitol.

10. The method of claim 6, wherein said maturation medium comprises about 5-20  $\mu$ M abscisic acid, about 30-100 gm/liter sucrose, about 1 gm/liter casein hydrosylate, and about 10 gm/liter mannitol.

11. The method of claim 8, wherein said embryo induction medium further comprises about 400 to 1700 mg/liter  $\text{NH}_4\text{NO}_3$  and about 1900 to 3500 mg/liter  $\text{KNO}_3$ .

12. The method of claim 6, wherein said poinsettia plant tissue explants are selected from the group consisting of immature embryos, mature embryos, shoot tips and stem segments.

13. The method of claim 6, wherein said selectable marker gene is selected from the group consisting of a neomycin phosphotransferase gene, a hygromycin phosphotransferase gene, a phosphinothricin gene, a dihydrofolate reductase gene, a 5-enolpyruvylshikimate-3-phosphate synthase gene, an acetohydroxyacid synthase gene, a chloramphenicol acetyltransferase gene, a 3'-adenylyltransferase gene, a gentamicin acetyltransferase gene, a streptomycin phosphotransferase gene, and an aminoglycoside-3'-adenyl transferase gene.

14. The method of claim 13, wherein said selectable marker gene is hygromycin phosphotransferase and said selection agent is hygromycin.

15. The method of claim 6, wherein said expression vector that comprises said second foreign gene further comprises a promoter, wherein said promoter is selected

030344-0334

from the group consisting of Cauliflower Mosaic Virus (CaMV) 35S promoter, the enhanced 35S promoter, the UBQ3 promoter, the UBQ10 promoter, the UBQ11 promoter, the UBQ14 promoter, the TEFA 1 promoter, the rolC promoter, and the Commelina Yellow Mottle Virus promoter, wherein the expression of said second foreign gene is under the control of said promoter.

16. The method of claim 15, wherein said promoter is selected from the group consisting of the CaMV 35S promoter, the enhanced 35S promoter, the UBQ3 promoter, and the UBQ10 promoter.

17. The method of claim 6, wherein the expression of said second foreign gene confers resistance to disease caused by an organism selected from the group consisting of virus, bacterium, fungus, and insect.

18. The method of claim 17, wherein said second foreign gene disrupts the function of said virus, and wherein said virus-disrupting gene is selected from the group consisting of viral coat protein, 2'-5' oligonucleotide synthetase, viral genome antisense RNA, and pokeweed antiviral protein.

19. The method of claim 6, wherein said second foreign gene confers resistance to an insect, and wherein said insect resistance gene is selected from the group consisting of tryptophan decarboxylase, lectin, and *Bacillus thuringiensis* toxin.

20. The method of claim 19, wherein said lectin is *Galanthus nivalis* lectin.

21. The method of claim 6, wherein said second foreign gene confers resistance to a bacterium or a fungus, and wherein said second foreign gene encodes a polypeptide selected from the group consisting of

20250404 1450580

Suk  
83

chitinase, a  $\beta$ -1,3-glucanase, ribosome-inactivating protein, lytic peptide, and plant defensin.

22. The method of claim 21, wherein said plant defensin is radish seed Rs-AFP2.

23. The method of claim 21, wherein said lytic peptide is selected from the group consisting of a magainin, PGL<sup>a</sup>, PGL, xenopsin, caerulein, cecropin, MSI-99, MSI-55, and D5-C.

24. The method of claim 6, wherein said second foreign gene is operatively linked with a DNA molecule encoding pea vicilin signal peptide.

25. The method of claim 23, wherein said magainin is magainin 1 or magainin 2.

26. The method of claim 6, wherein said transgenic poinsettia comprises an expression vector that further comprises a third foreign gene.

27. The method of claim 6, wherein said second foreign gene encodes chitinase, and wherein said third foreign gene encodes  $\beta$ -1,3-glucanase.

28. The method of claim 6, wherein said second foreign gene encodes magainin 2, and wherein said third foreign gene encodes PGL<sup>a</sup> or PGL.

29. The method of claim 6, wherein the expression of said second foreign gene confers insensitivity to ethylene, and wherein said second foreign gene encodes a mutated ethylene receptor.

30. The method of claim 29, wherein said mutated ethylene receptor gene is the *Arabidopsis etr-1* gene or a tomato *NR* gene.

31. The method of claim 6, wherein said second foreign gene is the *Vitreoscilla* hemoglobin gene.

32. The method of claim 6, wherein said second foreign gene is an isopentenyl transferase gene, wherein the expression of said isopentenyl transferase gene is under the control of a promoter of a senescence-associated gene.

33. The method of claim 32, wherein said promoter is the *Arabidopsis* SAG12 gene promoter.

34. The method of claim 6, wherein said second foreign gene encodes a polypeptide having a MADS box domain.

35. The method of claim 34, wherein said second foreign gene is selected from the group consisting of the *PLENA* gene, the *SQUAMOSA* gene, the *DEFICIENS* A gene, the *GLOBOSA* gene, the *APTELA1* gene, the *APETALA3* gene, the *AGAMOUS* gene, the *OsmADS24* gene, the *OsmADS45* gene, and the *OsmADS1* gene.

36. The method of claim 6, wherein said second foreign gene encodes a protein that modifies plant habit.

37. The method of claim 36, wherein said gene is the *OsmADS1* or *phyA* gene.

38. The method of claim 6, wherein said expression vector is introduced by microparticle bombardment.

39. A method for producing transgenic poinsettia plants, comprising the steps of:

- (a) incubating poinsettia plant tissue explants capable of producing reddish epidermal callus in callus induction medium;

0390344 0390344

Sub  
E4



- (b) culturing embryogenic callus produced on said callus induction medium in liquid embryo induction medium;
- (c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;
- (d) filtering the culture and culturing the filtrate on solid embryo induction medium;
- (e) culturing embryos produced on said embryo development medium on maturation medium;
- (f) culturing said embryos on callus induction medium;
- (g) culturing epidermal callus produced on said callus induction medium on embryo induction medium to form embryogenic callus;
- (h) introducing an expression vector into said embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
- (h') introducing two expression vectors into said embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;
- (i) culturing said transformed embryogenic callus on selection medium;
- (j) culturing said transformed embryogenic callus containing embryos on developmental medium;
- (k) culturing said transformed embryos on maturation medium;
- (l) recovering transgenic plants from said transgenic embryos.

40. The method of claim 39, wherein said callus induction medium comprises about 0.5-0.8 mg/liter 1-naphthalene acetic acid, about 0.2-0.4 mg/liter 6-

benzylaminopurine and about 1 gm/liter casein hydrosylate.

41. The method of claim 39, wherein said embryo induction medium comprises about 0.5-0.8 mg/liter 1-naphthalene acetic acid, about 0.2-0.4 mg/liter 6-benzylaminopurine and about 1 gm/liter casein hydrosylate.

42. The method of claim 39, wherein said developmental medium comprises about 0.05 mg/liter 6-benzylaminopurine, and about 10 gm/liter mannitol.

43. The method of claim 39, wherein said maturation medium comprises about 5-20  $\mu$ M abscisic acid, about 30-100 gm/liter sucrose, about 1 gm/liter casein hydrosylate, and about 10 gm/liter mannitol.

44. The method of claim 40, wherein said embryo induction medium further comprises about 400 to 1700 mg/liter  $\text{NH}_4\text{NO}_3$  and about 1900 to 3500 mg/liter  $\text{KNO}_3$ .

45. The method of claim 39, wherein said poinsettia plant tissue explants are selected from the group consisting of immature embryos, mature embryos, shoot tips and stem segments.

46. The method of claim 39, wherein said poinsettia plant tissue explants are selected from the group consisting of immature embryos, mature embryos, shoot tips and stem segments.

47. The method of claim 39, wherein said selectable marker gene is selected from the group consisting of a neomycin phosphotransferase gene, a hygromycin phosphotransferase gene, a phosphinothricin gene, a dihydrofolate reductase gene, a 5-enolpyruvylshikimate-3-phosphate synthase gene, an acetohydroxyacid synthase gene, a chloramphenicol acetyltransferase gene, a 3"-

08903944-073497



adenylyltransferase gene, a gentamicin acetyltransferase gene, a streptomycin phosphotransferase gene, and an aminoglycoside-3'-adenyl transferase gene.

48. The method of claim 47, wherein said selectable marker gene is hygromycin phosphotransferase and said selection agent is hygromycin.

49. The method of claim 39 wherein said expression vector that comprises said second foreign gene further comprises a promoter, wherein said promoter is selected from the group consisting of Cauliflower Mosaic Virus (CaMV) 35S promoter, the enhanced 35S promoter, the UBQ3 promoter, the UBQ10 promoter, the UBQ11 promoter, the UBQ14 promoter, the TEFA 1 promoter, the rolC promoter, and the Commelina Yellow Mottle Virus promoter, wherein the expression of said second foreign gene is under the control of said promoter.

50. The method of claim 49, wherein said promoter is selected from the group consisting of the CaMV 35S promoter, the enhanced 35S promoter, the UBQ3 promoter, and the UBQ10 promoter.

51. The method of claim 39, wherein the expression of said second foreign gene confers resistance to disease caused by an organism selected from the group consisting of virus, bacterium, fungus, and insect.

52. The method of claim 51, wherein said second foreign gene disrupts the function of said virus, and wherein said virus-disrupting gene is selected from the group consisting of viral coat protein, 2'-5' oligonucleotide synthetase, viral genome antisense RNA, and pokeweed antiviral protein.

53. The method of claim 39, wherein said second foreign gene confers resistance to an insect, and wherein said insect resistance gene is selected from the group

45 FEB 20 11 46 AM '80

Suh  
E5

-9- 101

consisting of tryptophan decarboxylase, lectin, and *Bacillus thuringiensis* toxin.

54. The method of claim 53, wherein said lectin is *Galanthus nivalis* lectin.

55. The method of claim 39, wherein said second foreign gene confers resistance to a bacterium or a fungus, and wherein said second foreign gene encodes a polypeptide selected from the group consisting of chitinase, a  $\beta$ -1,3-glucanase, ribosome-inactivating protein, lytic peptide, and plant defensin.

56. The method of claim 55, wherein said plant defensin is radish seed Rs-AFP2.

57. The method of claim 55, wherein said lytic peptide is selected from the group consisting of a magainin, PGL<sup>a</sup>, PGL, xenopsin, caerulein, cecropin, MSI-99, MSI-55, and D5-C.

58. The method of claim 39, wherein said second foreign gene is operatively linked with a DNA molecule encoding pea vicilin signal peptide.

59. The method of claim 39, wherein said magainin is magainin 1 or magainin 2.

60. The method of claim 39, wherein said transgenic poinsettia comprises an expression vector that further comprises a third foreign gene.

61. The method of claim 39, wherein said second foreign gene encodes chitinase, and wherein said third foreign gene encodes  $\beta$ -1,3-glucanase.

62. The method of claim 39, wherein said second foreign gene encodes magainin 2, and wherein said third foreign gene encodes PGL<sup>a</sup> or PGL.

20250704-110000

63. The method of claim 39, wherein the expression of said second foreign gene confers insensitivity to ethylene, and wherein said second foreign gene encodes a mutated ethylene receptor.

64. The method of claim 63, wherein said mutated ethylene receptor gene is the *Arabidopsis etr-1* gene or a tomato NR gene.

65. The method of claim 39, wherein said second foreign gene is the *Vitreoscilla* hemoglobin gene.

66. The method of claim 39, wherein said second foreign gene is an isopentenyl transferase gene, wherein the expression of said isopentenyl transferase gene is under the control of a promoter of a senescence-associated gene.

67. The method of claim 66, wherein said promoter is the *Arabidopsis SAG12* gene promoter.

68. The method of claim 39, wherein said second foreign gene encodes a polypeptide having a MADS box domain.

69. The method of claim 68, wherein said second foreign gene is selected from the group consisting of the *PLENA* gene, the *SQUAMOSA* gene, the *DEFICIENS A* gene, the *GLOBOSA* gene, the *APTELA1* gene, the *APETALA3* gene, the *AGAMOUS* gene, the *OsmADS24* gene, the *OsmADS45* gene, and the *OsmADS1* gene.

70. The method of claim 39, wherein said second foreign gene encodes a protein that modifies plant habit.

71. The method of claim 70, wherein said gene is the *OsmADS1* or *phyA* gene.

2025-11-14 14:00:00

72. The method of claim 39, wherein said expression vector is introduced by microparticle bombardment.

73. A transgenic poinsettia plant comprising at least one expression vector, wherein said expression vector comprises at least one foreign gene, and wherein said transgenic poinsettia plant expresses said foreign gene.

74. The transgenic poinsettia plant of claim 73, wherein said expression vector further comprises a promoter, wherein said promoter is selected from the group consisting of Cauliflower Mosaic Virus (CaMV) 35S promoter, the enhanced 35S promoter, the UBQ3 promoter, the UBQ10 promoter, the UBQ11 promoter, the UBQ14 promoter, the TEFA 1 promoter, the rolC promoter, and the Commelina Yellow Mottle Virus promoter, and wherein the expression of said foreign gene is under the control of said promoter.

75. The transgenic poinsettia plant of claim 74, wherein said promoter is selected from the group consisting of the CaMV 35S promoter, the enhanced 35S promoter, the UBQ3 promoter, and the UBQ10 promoter.

76. The transgenic poinsettia plant of claim 73, wherein the expression of said foreign gene confers resistance to disease caused by an organism selected from the group consisting of virus, bacterium, fungus, and insect.

77. The transgenic poinsettia plant of claim 76, wherein said foreign gene disrupts the function of said virus, and wherein said virus-disrupting gene is selected from the group consisting of viral coat protein, 2'-5' oligonucleotide synthetase, viral genome antisense RNA, and pokeweed antiviral protein.

446000-0000

Sub  
Ep

78. The transgenic poinsettia plant of claim 70, wherein said foreign gene confers resistance to an insect, and wherein said insect resistance gene is selected from the group consisting of tryptophan decarboxylase, lectin, and *Bacillus thuringiensis* toxin.

79. The transgenic poinsettia plant of claim 78, wherein said lectin is *Galanthus nivalis* lectin.

Sub 7 80. The transgenic poinsettia plant of claim 76, wherein said foreign gene confers resistance to a bacterium or a fungus, and wherein said foreign gene encodes a polypeptide selected from the group consisting of chitinase, a  $\beta$ -1,3-glucanase, ribosome-inactivating protein, lytic peptide, and plant defensin.

81. The transgenic poinsettia plant of claim 80, wherein said plant defensin is radish seed RS-AFP2.

82. The transgenic poinsettia plant of claim 80, wherein said lytic peptide is selected from the group consisting of a magainin, PGL<sup>a</sup>, PGL, xenopsin, caerulein, cecropin, MSI-99, MSI-55, and D5-C.

83. The transgenic poinsettia plant of claim 73, wherein said foreign gene is operatively linked with a DNA molecule encoding pea vicilin signal peptide.

84. The transgenic poinsettia plant of claim 82, wherein said magainin is magainin 1 or magainin 2.

85. The transgenic poinsettia plant of claim 73, wherein said transgenic poinsettia comprises an expression vector that comprises a second foreign gene.

86. The transgenic poinsettia plant of claim 85, wherein said foreign gene encodes chitinase, and wherein said second foreign gene encodes  $\beta$ -1,3-glucanase.

87. The transgenic poinsettia plant of claim 86, wherein said foreign gene encodes magainin 2, and wherein said second foreign gene encodes PGL<sup>a</sup> or PGL.

88. The transgenic poinsettia plant of claim 86, wherein the expression of said foreign gene confers insensitivity to ethylene, and wherein said foreign gene encodes a mutated ethylene receptor.

89. The transgenic poinsettia plant of claim 88, wherein said mutated ethylene receptor gene is the *Arabidopsis etr-1* gene or a tomato *NR* gene.

90. The transgenic poinsettia plant of claim 73, wherein said foreign gene is the *Vitreoscilla* hemoglobin gene.

91. The transgenic poinsettia plant of claim 73, wherein said foreign gene is an isopentenyl transferase gene, wherein the expression of said isopentenyl transferase gene is under the control of a promoter of a senescence-associated gene.

92. The transgenic poinsettia plant of claim 91, wherein said promoter is the *Arabidopsis SAG12* gene promoter.

93. The transgenic poinsettia plant of claim 73, wherein said foreign gene encodes a polypeptide having a MADS box domain.

94. The transgenic poinsettia plant of claim 93, wherein said foreign gene is selected from the group consisting of the *PLENA* gene, the *SQUAMOSA* gene, the *DEFICIENS A* gene, the *GLOBOSA* gene, the *APTELA1* gene, the *APETALA3* gene, the *AGAMOUS* gene, the *OsmADS24* gene, the *OsmADS45* gene, and the *OsmADS1* gene.

95. The transgenic poinsettia plant of claim 73, wherein the expression of said foreign gene modifies plant habit.

96. The transgenic poinsettia plant of claim 95, wherein said foreign gene is the *OSMADS1* or *phyA* gene.

add  
B4  
+C' - 28  
Add  
F10

20050604 14:40:26